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Effects of selenium supply and dietary restriction on maternal and fetal metabolic hormones in pregnant ewe lambs[1]

M. A. Ward,*[2] T. L. Neville,* J. J. Reed,* J. B. Taylor,† D. M. Hallford,‡ S. A. Soto-Navarro,‡ K. A. Vonnahme,* D. A. Redmer,* L. P. Reynolds,* and J. S. Caton*[3]

*Center for Nutrition and Pregnancy and Animal and Range Sciences Department, North Dakota State University, Fargo 58105; †USDA-ARS, US Sheep Experiment Station, Dubois, ID 83423; and ‡Animal and Range Sciences Department, New Mexico State University, Las Cruces 88003

ABSTRACT: The objective of these studies was to evaluate the effects of dietary restriction and Se on maternal and fetal metabolic hormones. In Exp. 1, pregnant ewe lambs (n = 32; BW = 45.6 ± 2.3 kg) were allotted randomly to 1 of 4 treatments. Diets contained (DM basis) either no added Se (control), or supranutritional Se added as high-Se wheat at 3.0 mg/kg (Se-wheat), or sodium selenate at 3 (Se3) and 15 (Se15) mg/kg of Se. Diets (DM basis) were similar in CP (15.5%) and ME (2.68 Mcal/kg). Treatments were initiated at 50 ± 5 d of gestation. The control, Se-wheat, Se3, and Se15 treatments provided 2.5, 75, 75, and 375 μg/kg of BW of Se, respectively. Ewe jugular blood samples were collected at 50, 64, 78, 92, 106, 120, and 134 d of gestation. Fetal serum samples were collected at necropsy on d 134. In Exp. 2, pregnant ewe lambs (n = 36; BW 53.8 ± 1.3 kg) were allotted randomly to treatments in a 2 × 2 factorial arrangement. Factors were nutrition (control, 100% of requirements vs. restricted nutrition, 60% of control) and dietary Se (adequate Se, 6 μg/kg of BW vs. high Se, 80 μg/kg of BW). Selenium treatments were initiated 21 d before breeding, and nutritional treatments were initiated on d 64 of gestation. Diets were 16% CP and 2.12 Mcal/kg of ME (DM basis). Blood samples were collected from the ewes at 62, 76, 90, 104, 118, 132, and 135 d of gestation. Fetal blood was collected at necropsy on d 135. In Exp.1, dietary Se source and concentration had no effect ($P > 0.17$) on maternal and fetal serum IGF-I, triiodothyronine (T_3), or thyroxine (T_4) concentrations. Selenium supplementation increased ($P = 0.06$) the T_4:T_3 ratio vs. controls. In Exp. 2, dietary Se had no impact ($P > 0.33$) on main effect means for maternal and fetal serum IGF-I, T_3, or T_4 concentrations from d 62 to 132; however, at d 135, high-Se ewes had lower ($P = 0.01$) serum T_4 concentrations than adequate-Se ewes. A nutrition by Se interaction ($P = 0.06$) was detected for the T_4:T_3 ratios; ewes fed restricted and adequate-Se diets had greater ($P = 0.10$) T_4:T_3 ratios compared with the other treatments. Nutrient-restricted ewes had lower ($P < 0.05$) serum IGF-I, T_3, and T_4 concentrations. Fetal serum IGF-I concentrations were lower ($P = 0.01$) in restricted- vs. control-fed ewes; however, fetal T_3 and T_4 concentrations were unaffected ($P > 0.13$) by dietary Se or maternal plane of nutrition. These data indicate that dietary Se may alter maternal T_4:T_3 ratios. In addition, nutrient restriction during gestation reduces maternal IGF-I, T_3, and T_4 and fetal IGF-I concentrations.

Key words: ewe, fetus, metabolic hormone, pregnancy, selenium

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INTRODUCTION

Selenium is a trace element that has diverse biological functions (Sunde, 1997; McDowell, 2003). In mammalian systems Se, via the selenoprotein type 1 iodothyronine 5′-deiodinase (Arthur et al., 1990), participates in the conversion of thyroxine (**T_4**) to triiodothyronine (**T_3**; Beckett et al., 1987). Circulating concentrations of T_3 and T_4 are reported to decline during nutrient restriction in cattle, geldings, and sheep (Hayden et al., 1993; Powell et al., 2000; Rae et al., 2002). Thyroid

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[2]Present address: Colby College, 1040 Golden St., Colby, KS 67701.

[3]Corresponding author: Joel.Caton@ndsu.edu

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hormones have been shown to be involved in the regulation of IGF-I (Thissen and Underwood, 1994); however, relationships between dietary Se and circulating concentrations of thyroid hormones and IGF-I remain unclear, particularly in ruminants. In addition, much of the available data relate to Se deficiencies rather than supranutritional levels. Reed et al. (2007) reported that nutrient restriction reduced and supranutritional Se supplementation increased lamb birth weights. These responses could be mediated through metabolic hormones. Although nutrient restriction impacts IGF-I in sheep and cattle (Hornick et al., 1998; Brameld et al., 2000), the interactions between nutrient restriction and dietary Se have not been published. In addition, selenomethionine is metabolized differently than inorganic sources of dietary Se (Thomson et al., 1982). Therefore, chemical forms as well as dietary concentrations of Se may elicit different responses in metabolic hormones. Concurrent comparisons between source and concentration of dietary Se, and their effects on IGF-I, T_3, and T_4 in pregnant ruminants are not available in the literature. In addition, the combined effects of dietary Se and nutrient restriction on fetal IGF-I, T_3, and T_4 are not well defined.

Therefore, we hypothesized that nutrient restriction and supranutritional levels of Se would alter serum concentrations of IGF-I, T_3, and T_4 in pregnant ewe lambs and their offspring.

MATERIALS AND METHODS

The North Dakota State University Institutional Animal Care and Use Committee approved animal care and use for this study.

Ewes were individually housed in 0.91- × 1.2-m pens in a temperature-controlled (12°C) and ventilated facility for the duration of each experiment. Lighting within the facility was automatically timed to mimic natural daylight patterns in Fargo, North Dakota.

Animals and Treatments

Experiment 1. Thirty-two pregnant Whiteface Western ewe lambs (initial BW 45.6 ± 2.3 kg) were allotted randomly to 1 of 4 dietary treatments in a completely randomized design. All ewes were housed in individual pens (0.91 × 1.2 m) in an indoor facility and were fed their diets individually. Treatments (initiated on d 50 ± 5 d gestation; approximately 9.5 mo of age) were control (0.1 mg/kg of Se), Se-wheat (3 mg/kg of Se), 3 mg/kg of selenate (**Se3**), and 15 mg/kg of selenate (**Se15**). The Se-wheat and Se3 diets provided 75 μg of Se/kg of BW, whereas the Se15 treatment provided 375 μg of Se/kg of BW. All diets contained 5% soybean hulls, 33.5% beet pulp, 2.5% soybean meal, 27% dehydrated alfalfa pellets, and 32% wheat (DM basis); however, the Se-wheat diet was formulated using a high-Se (9 mg/kg) wheat sourced from a seleniferous region near Pierre, South Dakota. Diets (DM basis) were similar in CP (15.5%) and ME (2.68 Mcal/kg) and were fed to meet or exceed NRC requirements (NRC, 1985). All diets were delivered in a complete pelleted form (0.48 cm diameter) and were fed twice daily. All ewes were provided free access to water and trace mineralized salt containing no added Se (American Stockman, Overland Park, KS). Daily feed offered was based on 2.5% BW (as fed), with BW measured every 14 d. For S3 and Se15 treatments, selenate was dissolved in tap water and applied as a liquid top-dress once daily.

Experiment 2. Twenty-one days before breeding, Whiteface Western ewe lambs (approximately 8.5 mo of age) housed at the USDA, ARS, US Sheep Experiment Station (Dubois, ID) were assigned randomly to Se treatments (6 vs. 80 μg of Se/kg of BW daily). Ewes were separated by Se treatment and group-fed within pens a basal diet consisting of 47% alfalfa hay, 20% corn, 20% sugarbeet pulp pellets, 8% malt barley straw, and 5% concentrated separator byproduct (DM basis) at a rate of 2.04 kg/ewe daily. In addition to the basal diet, each adequate-Se ewe received 100 g/d of a pellet containing 96% corn and 4% molasses, and each high-Se ewe received 100 g/d of a pellet containing 88% corn, 4% molasses, and 8% Se-enriched yeast (Sel-Plex, Alltech Inc., Nicholasville, KY) supplement mix. At approximately d 50 of gestation, ewes were pregnancy tested using ultrasound (Aloka, Tokyo, Japan). Pregnant ewes (n = 36) were shipped to the Animal Nutrition and Physiology Center at North Dakota State University for the remainder of the experiment.

Upon arrival at North Dakota State University ewes were individually housed and fed and the Se treatments continued. On d 64 of gestation, ewes (53.8 ± 1.3 kg of BW) were assigned randomly to 1 of 2 nutritional planes [100% (control) vs. 60% (restricted) of requirements for gestating ewe lambs (NRC, 1985)] within the Se treatments, resulting in a completely randomized design with 4 treatments arranged in a 2 × 2 factorial. Diets were individually fed once daily, with free access to water and trace mineralized salt (containing no added Se; American Stockman). Diets (Reed et al., 2007) were similar in CP (16.0%) and ME (2.12 Mcal ME/kg) and consisted of chopped alfalfa hay (3.8 cm in length), whole corn, and pelleted (0.48 cm diameter) supplements (as described above). Chopped alfalfa was top dressed with the supplement and corn. Individual feed ingredient samples were analyzed for DM, ash, N, (methods 930.15, 942.05, and 990.02, respectively; AOAC, 1990), ADF, and NDF (Ankom, Fairport, NY), and Se by atomic absorption spectroscopy (Finley et al., 1996). Diets were fed such that the total feed offered was consumed.

Nutrient requirements were based on the NRC (1985) recommendations for pregnant ewe lambs (60 kg of BW) in mid to late gestation (weighted ADG of 140 g). Intake of the respective supplements and corn were calculated based on BW, ME requirements, and the ME and Se concentrations of the supplement. Body weight was measured every 14 d.

Table 1. Maternal serum IGF-I, triiodothyronine (T_3) and thyroxine (T_4) concentrations (ng/mL), and T_4:T_3 ratios in pregnant ewe lambs fed different sources and concentrations of Se (Exp. 1)

	Treatment[1]						Contrasts[2]		
Item	CON	Se-wheat	Se3	Se15	SE	*P*-value[3]	CON vs. Se	Se-wheat vs. Se3	Se3 vs. Se15
IGF-I	272.8	266.3	235.7	248.0	19.0	0.50	0.31	0.26	0.65
T_3	2.6	2.3	2.5	2.5	0.1	0.37	0.22	0.31	0.88
T_4	91.9	88.1	95.7	103.9	4.1	0.07	0.40	0.20	0.17
T_4:T_3	38.5	42.4	42.2	43.1	1.8	0.29	0.06	0.94	0.71

[1]Control (CON; n = 56), selenium wheat (Se-wheat; 3 mg/kg; n = 56), selenate (Se3, 3 mg/kg, n = 56; and Se15, 15 mg/kg, n = 56). Treatments were applied from d 50 to 134 of gestation, and samples were collected at 7 periods during gestation, but treatment × sampling period interactions were not detected ($P > 0.10$); therefore, diet effects were examined across sampling period.

[2]Orthogonal contrasts were CON vs. Se-treated ewes, Se-wheat (3 mg/kg) vs. Se3 (3 mg/kg), and Se3 (3 mg/kg) vs. Se15 (15 mg/kg).

[3]Probability value for overall treatment *F*-test.

Sample Collection

Blood sample collection procedures were similar in Exp. 1 and Exp. 2; they varied only in the timing of the blood sampling and the interval relative to stage of gestation. In Exp. 1, jugular blood samples were collected from ewes at 50, 64, 78, 92, 106, 120, and 134 d of gestation, with fetal blood samples taken at d 134 (necropsy). In Exp. 2, jugular blood was taken from the ewes at 62, 76, 90, 104, 118, and 132 d of gestation, with maternal and fetal blood samples also taken at d 135 (necropsy). In both experiments, maternal blood (10 mL) was collected with Corvac serum separator vacuum tubes (Tyco Healthcare, Mansfield, MA) via jugular venipuncture and the serum harvested after centrifugation, as described below. In addition, 10 mL of blood was collected with sterile EDTA (K_3) Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). Fetal blood (10 mL, intracardiac) was collected into serum separator tubes as described above on d 134 (Exp.1) or d 135 (Exp. 2). All blood samples were placed on ice, held a minimum of 45 min, and then centrifuged at 1,500 × *g* for 30 min.

The supernatant was pipetted into 2-mL screw cap vials and stored at –20°C.

Serum Analysis

Serum IGF-I was quantified by RIA according to procedures of Berrie et al. (1995). Thyroxine and T_3 concentrations were determined by RIA utilizing components of commercial kits (Diagnostic Products Corp., Los Angeles, CA), with modifications described by Richards et al. (1999) for T_4 and Wells et al. (2003) for T_3. Serum IGF-I was determined in assays with intra- and interassay CV of 12.6 and 4.1%. Both T_3 and T_4 were measured in 2 assays each, with intraassay CV of 3.8 and 4.2%, respectively. The interassay CV for T_3 and T_4 were 2.5 and 4.2%, respectively.

Statistical Analyses

Experiment 1. Maternal and fetal serum metabolic hormone data were analyzed using PROC MIXED (SAS Inst. Inc., Cary, NC). Data from the ewes were analyzed as a split-plot design for repeated measures for d 50, 64, 78, 92, 106, 120, and 134 d of gestation. The model contained fixed effects for treatment, period, and treatment × period. Compound symmetry was used as the covariance structure, and ewe within treatment was used to test for treatment effects. Residual error was used for testing the effects of period and treatment × period. Fetal data were analyzed by ANOVA appropriate for a completely randomized design (GLM procedure of SAS). The incidence of twinning was 9.4%, and fetal number was included in the model. When significant ($P \leq 0.10$), fetal number was retained in the model. For the maternal model, ewe within treatment was used as the error term to test for treatment effects. When treatment by sampling time interactions were nonsignificant ($P > 0.10$), the main effects of treatment were separated using contrasts. Contrasts were used to evaluate differences between concentration and source of dietary Se; specifically, contrasts were made between control vs. Se treatments (Se-wheat, Se3, and Se15), Se-wheat vs. Se3, and Se3 vs. Se15.

The main effects of sampling time were evaluated using polynomial contrasts, which included linear, quadratic, and cubic effects. All polynomial contrasts were protected with a significant *F*-test for treatment ($P \leq 0.10$). Additional polynomial comparisons were possible but were not included, as they were of no interest in this study (Draper and Smith, 1980).

Experiment 2. Maternal hormone data were considered as a split-plot design with a 2 × 2 factorial arrangement of treatments in the main plot. Data were analyzed the using MIXED procedure of SAS for repeated measures, as described above (samples collected on d 62, 76, 90, 104, 118, and 132 d of gestation). The main plot included the 2 (control and restricted) × 2 (adequate

and high Se) factorial arrangement and the nutrition × Se interaction. Sampling day and its 2- and 3-way interactions with nutritional and Se concentrations were included in the subplot. Ewe within treatment was used as the error term to test the main plot effects. Ewe and fetal data from d 135 of gestation were analyzed as a completely randomized design. The incidence of twinning was 44.4%; therefore, fetal number was included in the model. When significant ($P \leq 0.10$), fetal number was retained in the model. The absence of nutrition × Se and nutrition × Se × day interactions ($P > 0.10$) allowed for presentation of main effect means. Data across sampling period (gestation day) were evaluated using polynomial contrasts and included linear, quadratic, and cubic effects. Contrasts were protected with a significant *F*-test for treatment ($P \leq 0.10$). Additional polynomial comparisons were possible, but were not included because they were of no interest in this study (Draper and Smith, 1980).

RESULTS

Experiment 1

As previously reported (Neville et al., 2008), Se treatments were effective in elevating plasma Se concentrations at necropsy (0.27, 0.55, 0.47, and 1.28 ± 0.06 mg/kg for control, Se-wheat, Se3, and Se15, respectively). Likewise, fetal BW was previously reported (Neville et al., 2008) to be similar among treatments evaluated here in Exp. 1.

Maternal serum IGF-I and T_3 concentrations were not affected ($P > 0.21$) by supranutritional Se supplementation, dietary source, or concentration of Se (treatment × sampling time, $P > 0.10$; Table 1). Contrasts for maternal serum T_4 were not different ($P > 0.16$) for Se supplementation, source of Se, and concentration of Se supplementation. Maternal T_4:T_3 ratio was increased ($P = 0.06$) by Se supplementation compared with control ewes. Fetal IGF-I, T_3, and T_4 concentrations (Table 2), and T_4:T_3 ratios were not different ($P > 0.20$) among treatments.

Serum IGF-I concentrations increased ($P < 0.001$) linearly in ewes from d 50 to 134 of gestation (Figure 1A). Other contrasts for IGF-I were also significant and included quadratic ($P < 0.08$) and cubic ($P < 0.001$) effects. The cubic effect was characterized by increasing IGF-I concentrations from d 50 to 78, declining concentrations from d 78 to 106, and then increasing from d 106 to 134. Conversely, maternal serum T_3 (Figure 1B) and T_4 (Figure 1C) concentrations decreased ($P < 0.001$) linearly with advancing day of gestation. Quadratic ($P \leq 0.05$) responses were also observed in these thyroid hormones; however, when observing the data in Figure 1A and B, the linear response is the most apparent and was also the most significant. Maternal T_4:T_3 ratio increased linearly ($P < 0.001$), as gestation progressed (Figure 1D). Quadratic and cubic ($P = 0.58$) effects were not present ($P > 0.16$) in maternal T_4:T_3 ratio with advancing gestation.

Experiment 2

As previously reported (Reed et al., 2007), nutrient restriction and dietary Se elevated plasma Se concentrations at necropsy (0.18, 0.40, 0.21, and 0.45 ± 0.02 mg/kg for control adequate-Se, control high-Se, restricted adequate-Se, and restricted high-Se, respectively). In addition, nutrient restriction has previously been reported to reduce fetal BW, whereas high-Se treatments used in the current study have previously been reported increase fetal BW (Reed et al., 2007).

No 2-way or 3-way interaction was detected ($P > 0.10$) among nutritional level, Se concentration, and sampling day for IGF-I, T_3, or T_4 (Table 3). Ewes fed 60% of control during the last two-thirds of gestation had reduced ($P = 0.01$) serum IGF-I concentrations (Table 3). Nutrient restriction also resulted in lower ($P < 0.05$) maternal serum T_3 and T_4 concentrations compared with control ewes. Similar to what was observed in Exp. 1, dietary Se concentration had no effect ($P = 0.72$) on maternal serum IGF-I. Dietary Se concentration had no effect ($P > 0.50$) on maternal serum T_3 and T_4 concentrations. Interestingly, there was a nutrition × Se interaction ($P = 0.06$) for maternal T_4:T_3 ratio. Interaction means for T_4:T_3 were 62.7, 62.7, 71.5, and 60.5 ± 3.3 ng/mL for control-adequate Se, control-high Se, restricted-adequate Se, and restricted-high Se, respectively. Ewes fed the restricted-adequate Se had elevated ($P < 0.10$) T_4:T_3 ratios compared with other treatments. Therefore, providing supranutritional levels of dietary Se to restricted ewes returned T_4:T_3 ratios to normal control concentrations (60.5 vs. 62.7 ± 3.3, respectively; $P > 0.10$).

Maternal serum IGF-I concentrations increased ($P < 0.002$) quadratically with increasing day of gestation (Figure 2A). Both maternal serum T_3 (Figure 2B) and T_4 (Figure 2C) concentrations declined linearly ($P < 0.001$) from d 62 to 132 of gestation. Quadratic responses ($P < 0.001$) were also present for maternal serum T_3 and T_4; however, when plotting the data, the most pronounced response for T_3 and T_4 was the linear decline with advancing gestation (Figure 2B and 2C). Because of a nutritional level × Se interaction ($P = 0.06$), maternal T_4:T_3 ratios were evaluated across advancing gestation within individual treatment. Ewe lambs fed a restricted diet and adequate Se had serum T_4:T_3 ratios of 59.8, 70.9, 77.1, 73.3, 72.4, and 71.3 ± 3.6 on d 62, 76, 90, 104, 118, and 132 of gestation (quadratic, $P = 0.001$). The T_4:T_3 ratios for the other 3 simple effect treatments were similar ($P > 0.13$) across sampling day (data not shown).

On the day of necropsy (135 ± 5 d of gestation) both maternal and fetal serum hormones responded to dietary treatment. Maternal serum IGF-I, T_3, and T_4 concentrations were lower ($P < 0.03$) in ewes fed 60% of maintenance compared with controls (Table 4). Serum T_4 concentrations were less ($P = 0.01$) at time of necropsy in ewes fed high dietary Se compared with ewes fed adequate dietary Se. Maternal serum T_4:T_3 ratios

Table 2. Fetal serum IGF-I, triiodothyronine (T_3) and thyroxine (T_4) concentrations (ng/mL), and T_4:T_3 ratios in pregnant ewe lambs fed different concentrations and sources of selenium (Exp. 1)

	Treatment[1]						Contrasts[2]		
Item	CON	Se-wheat	Se3	Se15	SE	*P*-value[3]	CON vs. Se	Se-wheat vs. Se3	Se3 vs. Se15
IGF-I	121.2	115.9	114.4	98.8	12.7	0.52	0.40	0.93	0.31
T_3	0.7	0.8	0.7	0.8	0.1	0.72	0.66	0.30	0.66
T_4	158.5	144.2	154.7	132.6	16.5	0.65	0.42	0.65	0.34
T_4:T_3	232.5	179.9	234.7	196.0	32.4	0.46	0.38	0.21	0.37

[1]Control (CON; n = 56), selenium wheat (Se-wheat; 3 mg/kg; n = 56), selenate (Se3, 3 mg/kg, n = 56; and Se15, 15 mg/kg, n = 56). Treatments were applied from d 50 to 134 of gestation.
[2]Orthogonal contrasts were CON vs. Se-treated ewes, Se-wheat (3 mg/kg) vs. Se3 (3 mg/kg), and Se3 (3 mg/kg) vs. Se15 (15 mg/kg).
[3]Probability value for overall treatment *F*-test.

at d 135 of gestation were not different between control vs. restricted or adequate Se vs. high Se treatments.

In the fetus, nutrient restriction decreased ($P < 0.01$) serum IGF-I concentrations compared with fetuses from control ewes (Table 4). Fetal serum T_3 concentrations were not altered ($P = 0.13$) as a result of nutrient restriction. Fetal serum T_4 concentrations and T_4:T_3 ratios were not different ($P > 0.60$) between main effect treatments.

DISCUSSION

In the current studies, the source and concentration of dietary Se supplementation had no effect on maternal and fetal serum T_3 and IGF-I concentrations. However, in Exp. 2 maternal serum T_4 concentrations were decreased by Se supplementation compared with ewes fed adequate Se. Maternal T_4:T_3 ratios were increased by Se supplementation in Exp. 1 and not in Exp. 2. Fetal T_4 concentrations and T_4:T_3 ratios were unaffected by source or concentration of Se treatment. In addition, no adverse or toxicological effects were observed in response to Se treatments used in this study.

The role of selenium in thyroid function was first demonstrated in rats and later in cattle and sheep (reviewed by Underwood and Suttle, 2001). Beckett et al. (1987) reported elevated T_4 and less circulating T_3 in rats depleted of dietary Se compared with rats receiving adequate Se. Arthur et al. (1988) reported that Se-deficient steers had poor conversion of T_4 to T_3. Selenium is a component of multiple deiodinases and thioredoxin reductases (reviewed by Beckett and Arthur, 2005; Kohrle et al., 2005), which highlights the importance of Se in thyroid hormone metabolism. Supplementing Se-deficient animals or humans with additional Se results in improved T_3 status (Beckett et al., 1993; Calomme et al., 1995; Hawkes and Keim, 2003). Awadeh et al. (1998) reported that dietary concentration of Se (organically bound and inorganic) of cattle in Se-marginal areas resulted in improved T_3 status of dams and calves. However, Behne et al. (1992) reported that additional Se from either selenite or high-Se yeast in the diet (0.3 vs. 2.0 mg/d) did not influence thyroid tissue T_3 or T_4 concentrations in rats. Similarly in Exp. 1 of this study, serum T_3 and T_4 concentrations were not affected by source or concentration of dietary Se in the

Table 3. Maternal serum IGF-I, triiodothyronine (T_3) and thyroxine (T_4) concentrations (ng/mL), and T_4:T_3 ratios as influenced by level of nutrition and dietary Se (Exp. 2)

	Nutrition[1]		Selenium[2]			*P*-value[3]		
Item	CON	RES	ASe	HSe	SE	Nut	Se	Se × Nut
IGF-I	179.5	138.9	156.4	161.9	10.6	0.01	0.72	0.66
T_3	0.9	0.7	0.8	0.8	0.03	0.01	0.51	0.36
T_4	51.8	46.0	49.6	48.2	2.1	0.05	0.66	0.30
T_4:T_3[4]	62.7	66.0	67.1	61.6	2.1	0.26	0.07	0.06

[1]Nutritional treatments (applied from d 60 to 135 of gestation) were control (CON) and restricted (RES, 60% of controls).
[2]Selenium treatments (applied from 21 d before breeding until d 135 of gestation) were daily intake of organically-bound Se; adequate Se (ASe, 6 μg/kg of BW) and high Se (HSe, 80 μg/kg of BW).
[3]Probability values for effects of nutrition (Nut), selenium (Se), and the interaction. Except for T_4:T_3, absence of nutrition × Se and nutrition × Se × sampling day interactions ($P > 0.10$) allowed for presentation of main effects.
[4]Interaction means for maternal the T_4:T_3 were 62.7, 62.7, 71.5, and 60.6 ± 3.3; for CON-ASe (n = 48), CON-HSe (n = 60), RES-ASe (n = 60), and RES-HSe (n = 42), respectively.

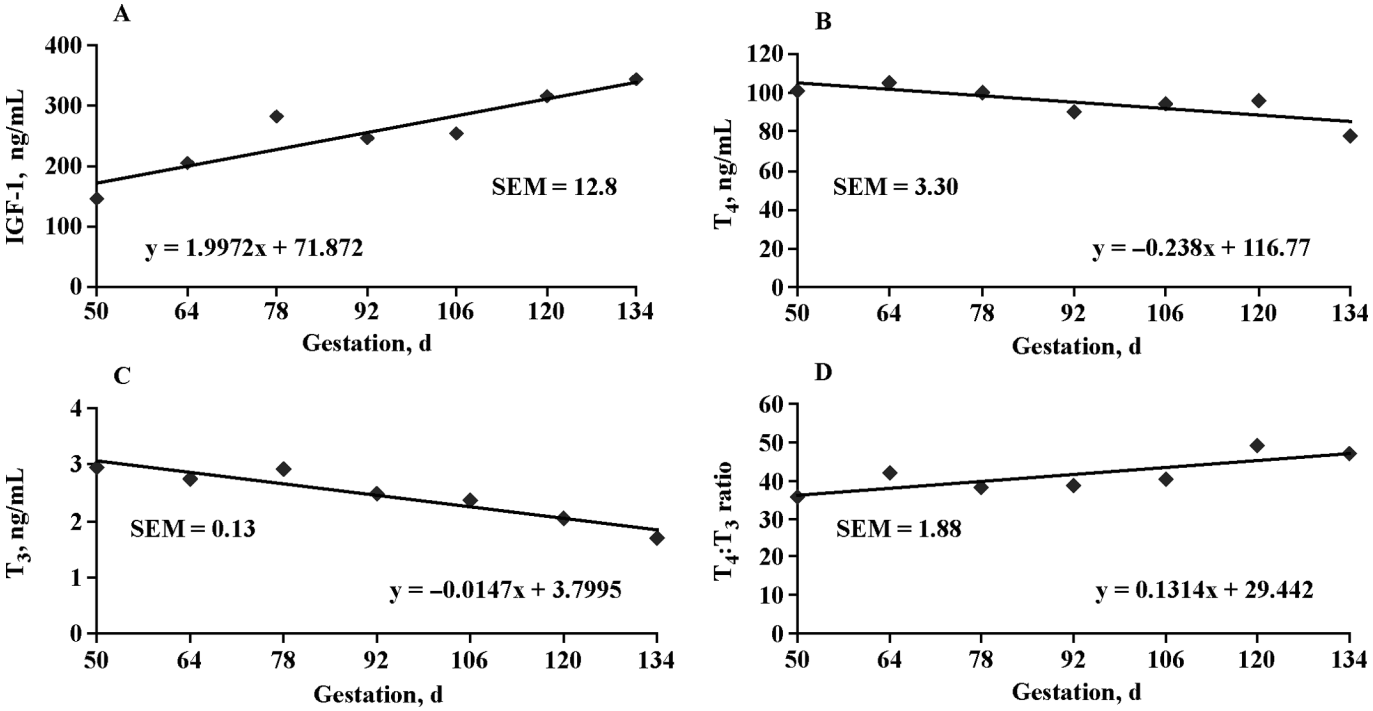

Figure 1. Effects of advancing gestation on serum A) IGF-I, B) triiodothyronine (T_3), and C) thyroxine (T_4) concentrations (D), and T_4:T_3 ratios in pregnant ewe lambs (Exp. 1). Treatment by sampling day interactions were not detected ($P > 0.10$). Linear responses across sampling day were observed for all hormones ($P < 0.001$).

pregnant ewe or their fetuses. However, maternal T_4:T_3 ratios (Exp. 1) were increased by Se supplementation in pregnant ewes. Elevated T_4:T_3 may indicate altered deiodinase activity as typically noted in Se-deficient animals (Beckett et al., 1987; Underwood and Suttle, 2001). The response in T_4:T_3 observed in the current study was unexpected considering none of the ewes were Se deficient and that Se is required for production of type I, II, and III deiodinase enzymes (Bates et al., 2000; Underwood and Suttle, 2001; Kohrle et al., 2005). Apparently, elevated dietary Se (supranutritional levels) may alter deiodinase activity in sheep. Additional work is needed evaluate this concept.

As in Exp. 1, dietary Se in Exp. 2 had no effect on overall maternal and fetal serum IGF-I, T_3, and T_4 concentrations. Similarly, Rowntree et al. (2004) reported

Table 4. Maternal and fetal serum IGF-I, triiodothyronine (T_3), and thyroxine (T_4) concentrations (ng/mL) and T_4:T_3 ratios on d 135 of gestation (necropsy), as influenced by level of nutrition and dietary Se (Exp. 2)

	Nutrition[1]		Selenium[2]			*P*-value[3]		
Item, ng/mL	CON	RES	ASe	HSe	SEM	Nut	Se	Se × Nut
Maternal								
IGF-I	142.8	77.4	109.4	110.8	12.3	0.01	0.94	0.87
T_3	0.74	0.58	0.70	0.62	0.04	0.01	0.16	0.91
T_4	44.5	38.2	45.0	37.6	2.0	0.02	0.01	0.22
T_4:T_3	60.8	66.7	66.2	61.4	2.9	0.14	0.24	0.26
Fetal								
IGF-I	78.7	60.8	66.6	72.9	4.8	0.01	0.33	0.36
T_3	0.35	0.31	0.33	0.33	0.02	0.13	0.94	0.68
T_4	105.4	102.3	104.4	103.2	4.6	0.62	0.85	0.91
T_4:T_3	307.8	326.2	318.2	315.8	15.2	0.37	0.90	0.35

[1]Nutritional treatments (applied from d 60 to 135 of gestation) were control (CON) and restricted (RES, 60% of controls).

[2]Selenium treatments (applied from 21 d before breeding until d 135 of gestation) were daily intake of organically bound Se; adequate Se (ASe, 6 μg/kg of BW) and high Se (HSe, 80 μg/kg of BW).

[3]Probability values for effects of nutrition (Nut), selenium (Se), and their interaction. Absence of nutrition × Se interactions ($P > 0.10$) allowed for presentation of main effects. Number of ewes and lambs were 8 and 12, 10 and 14, 10 and 16, and 7 and 9, respectively, for CON-ASe, CON-HSe, RES-ASe, and RES-HSe.

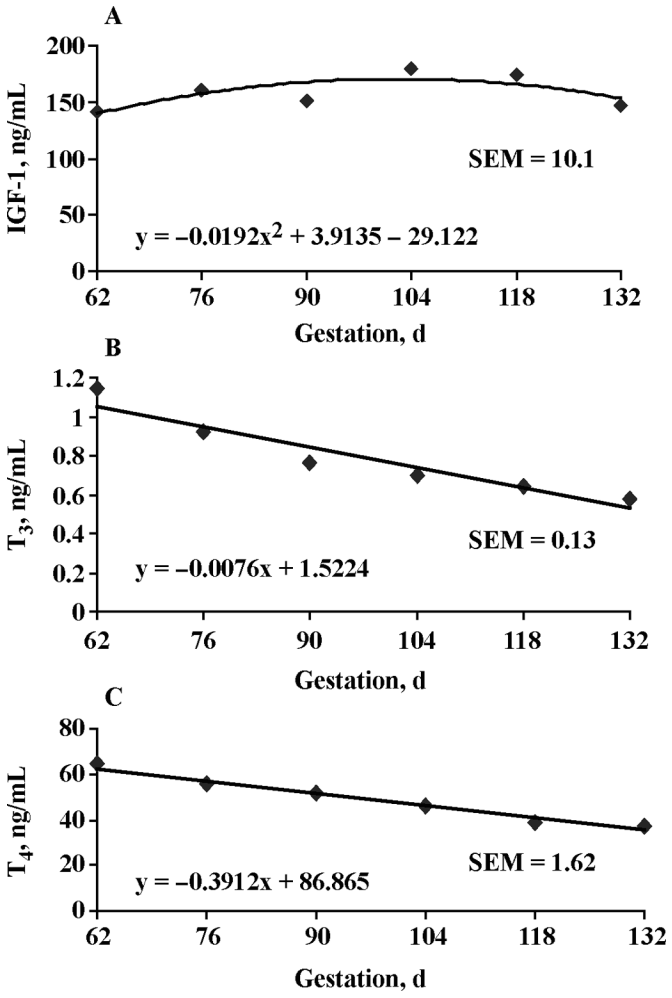

Figure 2. Effects of advancing gestation on serum A) IGF-I, B) triiodothyronine (T_3), and C) thyroxine (T_4) concentrations in pregnant ewe lambs (Exp. 2). Treatment by sampling day interactions were not detected ($P > 0.10$). Linear responses ($P < 0.003$) across sampling day were observed for T_3 and T_4, whereas a quadratic response ($P < 0.002$) was observed for IGF-I.

that maternal Se supplementation did not alter neonatal calf thyroid hormone concentrations. In the present study, however, maternal serum T_4 concentrations were less at necropsy (d 135) in ewes fed high dietary Se compared with controls.

In Exp. 2, a Se × nutrition interaction in maternal T_4:T_3 ratios was detected. Nutrient-restricted ewes receiving adequate Se had greater T_4:T_3 ratios compared with all other treatments. Additionally, Se supplementation to nutrient-restricted ewes retained maternal T_4:T_3 ratios at similar concentrations as nonrestricted ewes. Mechanisms associated with this response are unclear and perhaps merit additional investigation because Se supplementation alters T_3 concentrations and energy metabolism in men (Hawkes and Keim, 2003).

Little information is available regarding effects of dietary Se supplementation and circulating maternal and fetal IGF-I concentrations, and available data have varied. Moreno-Reyes et al. (2001) compared Se-deficient (0.005 mg/kg) diets with Se-adequate (0.19 mg/kg) diets and found a 50% reduction in plasma IGF-I concentrations in rats fed Se-deficient diets. Conversely, Gronbaek et al. (1995) reported a reduction in serum IGF-I concentrations in rats provided supranutritional levels (3.3 mg/L) in their water source compared with controls. Published studies mentioned above were in rapidly growing animals. In both experiments of our study, maternal serum IGF-I concentrations were not affected by source or concentration of dietary Se. When Se is sufficient in the diet, it appears that supranutritional dietary Se does not alter serum IGF-I in the pregnant ewe lamb. Additionally, serum IGF-I concentrations in the fetuses of the respective experiments were also unaffected by Se status of the dam. Therefore, effects of dietary Se on serum IGF-I concentrations seem to depend on species, age of animal, and reproductive status.

Nutrient restriction resulted in reduced maternal IGF-I, T_3, and T_4 concentrations. Conversely, in the fetus, only IGF-I was affected by maternal nutrient restriction, whereas dietary Se did not affect any of the metabolic hormones measured. Previous research indicates that nutrient restriction decreases circulating IGF-I in growing cattle and pregnant sheep (Hornick et al., 1998; Bispham et al., 2003). Additionally, increased dietary Se provided no benefit in circulating IGF-I concentrations in the nutrient-restricted ewes. Brameld et al. (2000) reported less IGF-I mRNA on d 80 of gestation in fetal livers from ewes fed 60% of maintenance. In their study, when ewes were realimented to maintenance diets, IGF-I mRNA abundance in the fetal liver was greater than in fetal livers from ewes fed to maintenance for the duration of gestation. In addition, Dong et al. (2005) reported that nutrient restriction (50% of controls) during early to mid gestation resulted in no change in fetal IGF-I, IGF-II, and IGF-binding protein 3 in fetal myocardium; however, they did report increased IGF-I and IGF-II receptor expression. Therefore, nutrient restriction appears to alter maternal metabolic hormones, while having minimal effects on fetal metabolic hormones.

In both Exp.1 and Exp. 2, maternal serum T_3 and T_4 concentrations declined linearly with advancing day of gestation. Other researchers, using pregnant goats, have shown that T_3 concentrations decline at parturition (Riis and Madsen, 1985). In Exp. 1, T_4:T_3 ratio increased linearly as gestation progressed. However, in Exp. 2, there was a nutrition × selenium interaction in T_4:T_3 ratios. In restricted ewes fed adequate Se, T_4:T_3 ratios responded both linearly and quadratically, with T_4:T_3 ratios increasing until d 90 of gestation and then declining. Perhaps deiodinase activity in this treatment was stimulated at the onset of the last third of pregnancy, allowing for more T_3 to be available for increased metabolic demand associated with the rapidly growing fetus. In restricted ewes fed high Se, T_4:T_3 ratio patterns as gestation advanced were not different from zero.

Maternal nutrition had little effect on fetal serum T_3 and T_4 concentrations; however, T_3 concentrations approached significance in fetuses from nutrient-restricted ewes. Serum concentrations of IGF-I declined linearly with advancing gestation in Exp. 1 and quadratically in Exp. 2.

In summary, when dietary Se is adequate, supranutritional Se supplementation does not alter maternal IGF-I, T_3, and T_4 during pregnancy. However, maternal T_4:T_3 ratios were greater in nutrient-restricted ewes compared with other treatments. Fetal metabolic hormones are not affected by supranutritional Se status in the dam. Ewes fed 60% of control during pregnancy have reduced serum IGF-I, T_3, and T_4 concentrations. Fetal serum IGF-I concentrations were also reduced when maternal nutrition was limiting, although fetal T_3 and T_4 were unaffected by maternal nutrition.

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